Journal of Molecular Graphics and Modelling 76 (2017) 466–474

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Molecular dynamics study of unfolding of lysozyme in water and its mixtures with dimethyl sulfoxide

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ARTICLE INFO

Article history:
Received 27 March 2017
Received in revised form 28 July 2017
Accepted 30 July 2017
Available online 1 August 2017

Keywords:
Molecular dynamics
Unfolding
Lysozyme
Tertiary structure
Secondary structure
Kinetics

ABSTRACT

All-atom explicit solvent molecular dynamics was used to study the process of unfolding of hen egg white lysozyme in water and mixtures of water with dimethyl sulfoxide at different compositions. We have determined the kinetic parameters of unfolding at a constant temperature 450 K. For each run, the time of disruption of the tertiary structure of lysozyme t_u was defined as the moment when a certain structural criterion computed from the trajectory reaches its critical value. A good agreement is observed between the results obtained using several different criteria. The secondary structure according to DSSP calculations is found to be partially unfolded to the moment of disruption of tertiary structure, but some of its elements keep for a long time after that. The values of t_u averaged over ten 30 ns-long trajectories for each solvent composition are shown to decrease very rapidly with addition of dimethyl sulfoxide, and rather small amounts of dimethyl sulfoxide are found to change the pathway of unfolding. In pure water, despite the loss of tertiary contacts and disruption of secondary structure elements, the protein preserves its compact globular state at least over 130 ns of simulation, while even at 5 mol percents of dimethyl sulfoxide it loses its compactness within 30 ns. The proposed methodology is a generally applicable tool to quantify the rate of protein unfolding in simulation studies.

1. Introduction

The rate and mechanism of denaturation of proteins may significantly vary with the changes in temperature, pressure, pH, ionic strength, and addition of various chemicals. Hen egg white lysozyme (HEWL) is a popular protein to study its denaturation under various conditions. Numerous works have been devoted to the thermodynamic and kinetic stability of lysozyme in pure water and in the presence of various additives and denaturants [1–5]. The significance of these works is connected with the advantages of aqueous-organic solvents as media for enzymatic processes and search for the ways to improve the stability of proteins in solutions.

Many common water-miscible organic solvents can lower the melting point of proteins. Dimethyl sulfoxide (DMSO, $(CH_3)_2SO$) is a low toxic solvent commonly used for solubilization of drugs and as a component of penetrating cryoprotectants for biological samples. Promotion of unfolding of lysozyme with DMSO was analyzed by means of differential scanning calorimetry (DSC) [6,7] in solution, which can provide the data on the temperature and enthalpy of denaturation and the heat capacities of the native and denatured forms of protein, and various spectroscopic techniques offering information on the changes in secondary and/or tertiary structure of protein upon unfolding. The maxima on DSC curves of HEWL corresponding to its melting point in solution steadily decrease with addition of growing amount of DMSO reaching room temperature at 35–50 mol percent of DMSO (depending on pH of the buffer). CD spectra indicate the loss of secondary and tertiary structure of lysozyme upon thermal unfolding in pure water [8]. The presence of DMSO leads to a strong absorption in the far UV region limiting the possibilities of this method. Small angle neutron scattering study of solution of lysozyme in the mixtures of pure water with DMSO at room temperature [9] has shown that it unfolds into a molten-globule like state with the loss of tertiary and partial preservation of the secondary structure in the range of DMSO mole fractions 0.37–0.7, while higher concentrations lead to complete unfolding into a random coil. Kinetics of thermal denaturation and renaturation of lysozyme in water has also been studied experimentally, and the possibility of intermediate formation was discussed. Some researchers suggest all-or-none model of lysozyme unfolding denying the existence of sufficiently stable intermediates [10], while others prove the existence of intermediates [11] or reversibly and irreversibly denatured forms in solution [12]. It is known that addition of organic solvents can alter the pathway of denaturation, and change the relative rates of disruption of secondary and tertiary structures [13]. The effect of particular denaturants on the mech-

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http://dx.doi.org/10.1016/j.jmgm.2017.07.032
1093-3263/